

Quantification of *Campylobacter* Species Cross-Contamination during Handling of Contaminated Fresh Chicken Parts in Kitchens

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Numerous outbreak investigations and case-control studies for campylobacteriosis have provided evidence that handling *Campylobacter*-contaminated chicken products is a risk factor for infection and illness. There is currently extremely limited quantitative data on the levels of *Campylobacter* cross-contamination in the kitchen, hindering risk assessments for the pathogen commodity combination of *Campylobacter* and chicken meat. An exposure assessment needs to quantify the transfer of the bacteria from chicken to hands and the kitchen environment and from there onto ready-to-eat foods. We simulated some typical situations in kitchens and quantified the *Campylobacter* transfer from naturally contaminated chicken parts most commonly used in Germany. One scenario simulated the seasoning of five chicken legs and the reuse of the same plate for cooked meat. In another, five chicken breast filets were cut into small slices on a wooden board where, without intermediate cleaning, a cucumber was sliced. We also investigated the transfer of the pathogen from chicken via hands to a bread roll. The numbers of *Campylobacter* present on the surfaces of the chicken parts, hands, utensils, and ready-to-eat foods were detected by using Preston enrichment and colony counting after surface plating on Karmali agar. The mean transfer rates from legs and filets to hands were 2.9 and 3.8%. The transfer from legs to the plate (0.3%) was significantly smaller ($P < 0.01$) than the percentage transferred from filets to the cutting board and knife (1.1%). Average transfer rates from hands or kitchen utensils to ready-to-eat foods ranged from 2.9 to 27.5%.

Most of the case-control studies and outbreak investigations for infections with the zoonotic bacteria *Campylobacter jejuni* and *C. coli* have identified consumption and handling of raw chicken as an important risk factor for human illness (1, 2, 8, 10, 13). Consumers' exposure to *Campylobacter* takes place either through consumption of undercooked, internally contaminated chicken meat or via cross-contamination to ready-to-eat food during the preparation of externally contaminated chicken parts and carcasses. There are currently limited quantitative data on *Campylobacter* cross-contamination available in the literature. Quantification of the transfer of *Campylobacter* from chicken via hands and the kitchen environment to ready-to-eat foods during handling of contaminated products is a central part of all risk assessments for this pathogen commodity combination. Using naturally contaminated fresh chicken parts purchased in retail stores, experiments were performed that simulated some of the typical situations and handling procedures that are common in German kitchens and presumably in many other countries as well.

MATERIALS AND METHODS

Sampling and quantification of *Campylobacter* on chicken parts. From June to November 2004 we bought random, independent packages containing several fresh chicken legs (drumstick plus thigh) or fresh, skinless and boneless chicken breast filets at various supermarkets in Berlin, Germany. Preliminary experiments have shown that all parts in a single package have approximately equal levels of bacterial load (K. Scherer, unpublished data). Thus, we quantified campylobacters on one leg or filet and used another piece of the same package for a transfer experiment, assuming it carried the same number of bacteria. To

quantify *Campylobacter* spp. on the surface of chicken legs, we used a 25-g skin sample (almost the whole skin of the leg), which was 1:10 diluted in Preston broth (CM 67 plus selective supplements SR204, SR232, and SR48; Oxoid GmbH, Wesel, Germany), and homogenized it for 2 min in a stomacher blender. The basis for quantification on the filets' surface was a 100-ml rinse sample. Enumeration was performed with Preston broth as diluent and Karmali agar (Oxoid CM935 plus SR205) for colony counting, as described previously (15, 23; P. Luber, P. Vogt, K. Scherer, and E. Bartelt, Proc. EU-RAIN Conf. Epidemiol. Zoonoses, abstr. P7, 2004). Isolates were identified to species level on the basis of phase-contrast microscopy (characteristic morphology and motility), Gram stain, catalase and oxidase production, growth at 25 and 43°C, indoxyl acetate hydrolysis, hippurate hydrolysis, and susceptibility to nalidixic acid and cephalothin.

Cross-contamination scenarios. Three different cross-contamination scenarios were simulated. Since it was not known in advance whether the chicken was contaminated, all experiments used five chicken parts from five different packages, and summing the numbers found on the surface of the five distinct parts generated the input number of *Campylobacter* for each cross-contamination experiment. Each transfer experiment started with the performing person washing his or her hands with soap and drying them with paper towels. All kitchen items used were autoclaved before each experiment.

Scenario 1 mimics the preparation of chicken legs for barbecuing and placing cooked meat on the same plate used for the raw meat. The person simulating the preparation touched five chicken legs which were taken from five different packages. The legs were placed on a ceramic plate (700 cm²). The chicken pieces were turned once to simulate the amount of handling, e.g., for seasoning, and left on the plate for 10 min. Eleven experiments were performed. In four of these, the surface of the plate was sampled directly after removal of the chicken legs. In the remaining seven a fried sausage was put on the plate after the removal of the raw chicken, to simulate the reuse of the same plate for raw chicken and cooked meat. Scenario 2 simulates the slicing of raw salad ingredients (cucumber) without washing hands, cutting board, or utensils after chicken breast filets are sliced on a wooden board. The person took five chicken breast filets from separate packages and placed them on a wooden chopping board (325 cm²) for slicing. After removal of the filets' slices, some slices of cucumber were cut using the same knife as used for the chicken. Eleven experiments were performed. Scenario 3 shows the direct transfer of *Campylobacter* from the chicken parts to hands and further to a bread roll, as an example of a ready-to-eat food. Four experiments were performed where participants handled five breast filets and

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TABLE 1. Amounts of dilution and rinsing fluids used for enumeration of *Campylobacter* spp. on hands, utensils, and ready-to-eat foods, including detection limits, lowest countable numbers, and amount of bacteria in case of positive enrichment only

Location	Vol of Preston broth used for rinsing or dilution	Lowest countable no. (CFU) of <i>Campylobacter</i> organisms	No. (CFU) of <i>Campylobacter</i> organisms in case of positive enrichment only	Detection limit (CFU)
Hands	10 ml of Maximum Recovery Diluent in 90 ml of Preston broth	100	10–99	<10
Plate	50 ml	50	1–49	<1
Sausage	100 ml	100	1–99	<1
Wooden board	Swabs immersed in 20 ml	20	1–19	<1
Knife blade	50 ml	50	1–49	<1
Cucumber cuts	25 g diluted in 225 ml	10	1–9	<1
Bread roll	100 ml	100	1–99	<1

one experiment where five legs were handled first before grasping a bread roll with unwashed hands.

At the end of each scenario, both hands of the person were sampled for *Campylobacter* by rinsing them for 30 s in a sterile plastic bag containing 100 ml of Maximum Recovery Diluent (Merck, Darmstadt, Germany). The first 25 g of cut cucumber slices was diluted and blended in 225 ml of Preston broth. All kitchen utensils, contact surfaces, and the bread roll and sausage were rinsed with Preston broth. For direct counting, duplicates of 1 ml of rinsing or dilution fluids were plated out on three plates of Karmali agar each. In addition, duplicates of 0.1 ml of fluid were plated out. To lower the threshold of measurement, all Preston broth rinsing and dilution fluids were microaerobically incubated for 24 h at 42°C, and 1 loopful (10 µl) of broth was streaked out on Karmali agar. Details on detection limits and volumes of Preston broth used for rinsing or dilutions are given in Table 1. All agar plates were incubated at 42°C for 48 h in a microaerobic atmosphere. Typical colonies were confirmed as *Campylobacter* species, and counted. The plates' surface was flushed with 50 ml of Preston broth, and the wooden board was sampled by swabbing. Swabs were thoroughly shaken in a tube holding 20 ml of Preston broth. The blade of the knife, the sausage, and the bread roll were sampled by rinsing each in a plastic bag with 50 or 100 ml of Preston broth.

Data analysis. Total bacterial counts for the five chicken parts used in transfer experiments, and total *Campylobacter* counts of hands, kitchen utensils, and ready-to-eat foods were determined. All numbers for samples with a positive enrichment only were set to a mean number of CFU (1 to 9 = 5, 1 to 19 = 10, 1 to 49 = 25, 1 to 99 = 50, and 10 to 99 = 55) for further calculations. The appropriate transfer rates were calculated (percent transfer rate = CFU on target/CFU on source × 100%) and the mean, standard deviation, and median were determined. Transfer rates from chicken breast filets and legs to hands, from filets and legs to kitchen utensils, and from hands and utensils to ready-

to-eat foods were analyzed for significant differences by means of the Mann-Whitney-test (14). Transfer rates from filets or legs to hands and utensils were investigated for significant differences. Statistical analysis was performed by using SPSS 12.0 software.

RESULTS

At least three of the five legs or breast filets used in each transfer experiment carried *Campylobacter* on their surface. Tables 2 to 4 present the counting results and calculated percent transfer rates for *Campylobacter* in the three different scenarios. Statistical measures (mean, standard deviation, and median) for the percent transfer rates are also given in each table.

Comparison of the rates of campylobacters that were transferred from legs or filets to hands (Tables 2 and 3) showed no statistical significant differences ($P > 0.05$). In contrast, the transfer rate for campylobacters from the surface of chicken breast filets to kitchen utensils (Table 3) was significantly ($P < 0.01$) larger than the rate for campylobacters transferred from chicken legs to a plate (Table 2). Although transfer rates for *Campylobacter* from kitchen utensils or hands to ready-to-eat foods such as a fried sausage, cucumber slices, or bread had a wide spread in the means (27.5, 10.3, and 2.9%, respectively),

TABLE 2. Transfer of campylobacters from the surface of chicken legs to hands and to a plate as observed in scenario 1^a

Sum of campylobacters (CFU) on five chicken leg surfaces	No. of <i>Campylobacter</i> organisms on:			Percentage of <i>Campylobacter</i> organisms transferred from:		
	Both hands (CFU/hands)	Plate (CFU/plate)	Sausage (CFU/sausage)	Chicken legs to hands	Legs to plate	Plate to a fried sausage
10,625	2,090	60	ND	19.7	0.6	
54,125	1,681	409	ND	3.1	0.8	
165,125	863	205	ND	0.5	0.1	
166,910	3,454	886	ND	2.1	0.5	
24,250	1,091	125	1–99	4.5	0.7	28.6
59,250	100	200	<1	0.2	0.3	0.0
204,375	636	227	1–99	0.3	0.1	18.1
104,975	545	50	1–99	0.5	0.1	50.0
48,125	100	100	1–99	0.2	0.3	33.3
17,900	100	1–49	<1	0.6	0.2	0.0
99,000	1,500	1–49	1–99	1.5	0.1	62.5
Mean				2.9*	0.3	27.5
SD				5.5*	0.3	23.7
Median				1.1*	0.3	28.6

^a Data on the transfer of bacteria from the plate to a fried sausage for seven experiments are provided. *, statistical data were calculated including the transfer rate from legs to hands as determined in scenario 3. ND, not determined.

TABLE 3. Results for scenario 2: transfer of campylobacters from the surface of five chicken breast filets during slicing to hands, the chopping board, and the blade of the knife and to cuttings of a cucumber, which was sliced directly after the breast filets^a

Sum of campylobacters (CFU) on five breast filets	No. of <i>Campylobacter</i> organisms on:				Percentage of <i>Campylobacter</i> organisms transferred from:		
	Both hands (CFU/hands)	Wooden board (CFU/board)	Knife blade (CFU/blade)	Cucumber cuts (CFU/25-g cuts)	Filets to hands	Filets to board and knife	Board and knife to cucumber
4,050	10–99	15	1–49	<1	1.2	0.9	0.0
5,150	10–99	<1	50	1–9	1.0	1.1	9.1
6,090	100	20	75	1–9	1.6	1.6	5.0
5,000	<10	1–19	75	10	0.2	1.9	10.5
15,000	10–99	1–19	1–49	1–9	0.3	0.2	14.3
2,800	100	1–19	50	1–9	3.6	2.3	7.7
1,600	<10	1–19	<1	1–9	0.6	0.9	33.3
13,900	1,090	20	1–49	10	7.8	0.4	20.0
6,120	150	20	50	1–9	2.5	1.2	6.7
13,300	350	20	<1	<1	2.6	0.2	0.0
5,500	10–99	20	50	1–9	0.9	1.4	6.7
Mean					3.8*	1.1	10.3
SD					5.9*	0.7	9.6
Median					2.5*	1.1	7.7

^a *, statistical data were calculated, including the transfer rates from filets to hands determined in scenario 3 (four experiments).

the observed differences are not statistically significant ($P > 0.05$). The difference in mean transfer rates from chicken legs to hands (2.9%) and the plate (0.3%) is significant ($P = 0.029$), whereas the transfer rates from filets to hands (3.8%) and from filets to kitchen utensils (1.1%) were not ($P > 0.05$).

DISCUSSION

The limited availability of relevant quantitative data has made the results of assessments of the risk of campylobacteriosis through chicken consumption very uncertain. Exposure assessment is a vital part of risk assessments, and there are currently almost no data that are specific for the transfer of *Campylobacter* from fresh chicken products to the environment and to ready-to-eat foods. Most transfer experiments have been performed with inoculated products with high numbers of an indicator bacterium (see, for example, references 3 and 26). The approach selected here uses naturally contaminated chicken and provides realistic data on the transfer of *Campylobacter* in the food preparation environment. Since even low doses of *Campylobacter* are known to lead to illness (19), a special effort was made to use methods that are able to detect contamination as low as 1 or 10 CFU *Campylobacter* per source or target of transfer.

Raw fresh chicken parts, in particular chicken legs (consisting of drumstick and thigh) and skinless and boneless breast filets, are the poultry products consumed most often by German households (25). Since heat treatment will kill off any campylobacters that are left on the surface (9), human exposure is dependent on the frequency and level of cross-contamination in the food preparation environment (5). Various scenarios of hygienic failures were simulated to give a picture of the various routes of potential exposure: from a direct contact between contaminated hands and mouth (21) to cross-contamination of ready-to-eat foods in the kitchen. The reuse of a plate and the reuse of cutting boards that had been used for raw chicken with a ready-to-eat product are two of the typical

scenarios for cross-contamination that were examined. In addition, the direct contamination from unwashed hands after preparing chicken directly to ready-to-eat foods, such as a bread roll, was examined. The need for realistic transfer data is apparent when the risk assessment models for campylobacteriosis are examined that have incorporated cross-contamination events during final preparation in a kitchen environment. A Danish risk assessment for *Campylobacter* in chicken (4, 20) restricted the consumer model only to focus on the effect of not washing the cutting board. The FAO/WHO draft risk assessment of *Campylobacter* in broiler chickens (7) models exposure of the consumer via undercooking and additionally addresses cross-contamination. Cross-contamination is simulated by use of a “drip fluid model.” The authors of that study do not explicitly specify routes of exposure but model the ingestion of volumes of chicken drip fluid, which contains loosely attached *Campylobacter* spp.

It has been shown that there are differences in the behavior of inoculated bacteria and surrogate bacteria compared to naturally found bacteria in foods and on chicken carcasses (18, 24). The study of transfer rates of *Campylobacter* with experiments using inoculated chicken skin samples, as described, for example, by Kusumaningrum et al. (11, 12), has disadvantages as well, since there is evidence that the use of high numbers of *Campylobacter* for inoculation leads to biased transfer rates (16). The use of surrogate bacteria or high levels of inoculated bacteria in transfer experiments should be critically evaluated when used, since they do not realistically reflect the exposure of the consumer to the pathogenic bacteria.

The raw data for our transfer experiments are provided to enable others to use these data sets for construction of new predictive cross-contamination models. In contrast to the recommendations given by Schaffner (22), we made no log transformation of the percent transfer rates before we calculated averages. As can be seen clearly in Tables 2 to 4, the distributions of percent transfer rates are right-skewed and distinctly nonnormal. Nevertheless, a statistical comparison of data sets

TABLE 4. Transfer of campylobacters from the surface of five breast filets or five chicken legs via hands to bread (scenario 3)

Sum of campylobacters (CFU)	No. of <i>Campylobacter</i> organisms on:		Total no. of campylobacters on hands and bread roll (CFU)	% of <i>Campylobacter</i> organisms transferred from:	
	Both hands (CFU/hands)	Bread roll (CFU/roll)		Filets or legs to hands	Hands to bread
On five breast filets					
13,900	2,000	1,318	3,318	23.9	9.5
6,120	100	1–99	101–199	2.5	0.8
13,300	455	250	705	5.3	1.9
5,500	10–99	100	110–199	2.7	1.8
On five legs					
99,000	1,090	410	1,500	1.5	0.4
Mean					2.9
SD					3.8
Median					1.8

can be performed by using the Mann-Whitney U-test (14). The use of empirical distributions in predictive modeling of the cross-contamination events will allow the full utilization of data.

Statistical analysis revealed significant differences between the amount of campylobacters that were transferred from the breasts and legs, with higher levels of transfer from the chicken breast filets to kitchen utensils ($P < 0.01$) and slightly higher levels to hands, than from the surface of a chicken leg ($P = 0.029$). The differences could either stem from the difference in product surfaces (with or without skin), the surfaces of the utensils, the amount of handling during preparation, or a combination of these factors. The chicken breasts were pressed during the slicing of the filet while the chicken legs were “placed and patted.” The differences in transfer rates can be explained by the degree of handling, but we cannot rule out that the differences in materials (ceramic, wood, and steel) influence the transfer of *Campylobacter* from the chicken surface to utensils. Only one typical procedure was simulated for each product. More experiments are needed to clarify causes for observed variations and future experiments should, e.g., additionally analyze the transfer of campylobacters from filets to a ceramic plate and from chicken legs to a wooden board. This would show if the observed differences are caused by variations in attachment of the pathogens to the surface of chicken parts with or without skin, or the nature of contact to the materials.

A qualitative cross-contamination study from The Netherlands showed that *C. jejuni* are easily transferred from raw chicken products to cutting boards, plates, and especially to hands (6). Cogan et al. (5) quantified cross-contamination in a volunteer study where the participants were asked to cut a naturally *Campylobacter*-contaminated whole raw chicken carcass into pieces. The results were that 85% of hands and 80% of cutting boards were contaminated, with 20% of the hands and 45% of the cutting boards at levels $>1,000$ CFU. This is difficult to relate directly to Germany, where the majority of chicken meat on the market is already in parts, but does correlate with more extensive contact giving higher levels of trans-

fer. The experiments here revealed high counts of *Campylobacter* on the hands (Tables 2 to 4). The importance of contaminated kitchen utensils compared to unwashed hands as a vehicle for transfer of campylobacters leading to exposure will depend to a great extent on consumer behavior in the kitchen, as well as the rates of ready-to-eat food prepared or handled after the chicken is prepared. The percentage of consumers that actually do wash their hands and/or utensils after handling raw meat, as well as the efficiency of the washing procedures, needs to be included in evaluating the exposure routes.

In a presentation of a model for cross-contamination during food preparation that was developed for the Dutch *Campylobacter* Risk Management and Assessment Project CARMA (S. Mylius, M. Nauta, and A. Havelaar, abstract and poster presented at CHRO 2003 in Aarhus, Denmark [Int. J. Med. Microb. 293, Suppl. 35, p. 28]) and that concentrates on the case of *Campylobacter*-contaminated chicken breast filets, high numbers of *Campylobacter* on hands were also reported. However, based on the results of model simulations the authors conclude that kitchen utensils may have more impact on cross-contamination leading to consumer exposure than hands.

Rates for further transfer of *Campylobacter* from kitchen utensils or hands to ready-to-eat foods such as cucumber, a fried sausage and bread varied, but were not statistically different. The only quantitative data for the transfer of *Campylobacter* published is from stainless steel surfaces to ready-to-eat-foods where mean transfer rates of 42.5% to cucumber slices (11) and of 16 to 38% for dry lettuce and 15 to 27% for wet lettuce were reported (17). Mean transfer rates to ready-to-eat foods determined in our experiments ranged from 2.9 to 27.9% and give a good indication on the variability of the different surface cross-contamination levels that can be expected in a varied kitchen environment.

The study reported here quantifies *Campylobacter* transfer rates, and the associated variability, from the most commonly consumed chicken parts to kitchen utensils and hands and the further transfer of the pathogen to ready-to-eat foods during handling of contaminated products in the kitchen. The use of naturally contaminated chicken and different scenarios gives a good basis for input data into risk assessment models, so that the influence of the cross-contamination routes in the kitchen on the level of human illness can be evaluated.

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